S10.P22

Transport of pyruvate into the mitochondrion of *Trypanosoma brucei*

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Trypanosoma brucei is a pathogen of livestock and humans transmitted by tse-tse flies in sub-Saharan Africa. Different life-cycle stages of trypanosomes present adaptations to their specific environment. In bloodstream and procyclic *T. brucei*, these include changes to mitochondrial morphology and function, and overall metabolic rearrangements reflected by different spectra of metabolic end products. In bloodstream *T. brucei*, ATP is generated primarily by glycolysis, pyruvate being the predominant excreted product of metabolism. In contrast, procyclic-stage *T. brucei*, found in the midgut of the insect vector where glucose is scarce, depend on mitochondrial catabolic pathways for ATP production. Proline and threonine are candidate carbon sources for these stages. In vitro, these are eventually metabolized to succinate, acetate and glycine. Regulating the availability of pyruvate in the mitochondrion is one of the modes of balancing oxidative phosphorylation and glycolysis; in bloodstream *T. brucei* this balance is shifted heavily towards glycolysis. We seek to determine whether *T. brucei* transports pyruvate into the mitochondrion using a mitochondrial pyruvate carrier homologous to the one recently identified in fruit fly, human and yeast cells (MPC). In addition, we address the relative importance of the pyruvate transporter in procyclic and bloodstream trypanosomes. To this end, we identified two MPC homologs in the genome *T. brucei* and confirmed the mitochondrial localization of the epitope-tagged proteins in both procyclic and bloodstream stages. We generated MPC1 knock-out cell lines in both these stages, showing that the pyruvate transporter is dispensable for *T. brucei* under standard culture conditions. The adaptations of mitochondrial metabolism in this model, as documented by end product analysis using HPLC and NMR, let us comment on mitochondrial metabolism in *T. brucei* in general.

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Role of formate channel coding focA and focB genes in H_2 production by *Escherichia coli* upon glucose fermentation at slightly alkaline pH

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Escherichia coli is able to encode four [Ni–Fe] hydrogenases (Hyd) having different roles in H_2 production and oxidation during glucose and/or glycerol fermentation that is dependent on pH [1]. Moreover, *E. coli* has two formate channels coded by focA and focB genes which are situated at the end of hyc and hyf operons, respectively. FocA has an important role in regulating intracellular formate level during anaerobic fermentation [2]. Role of focB gene is not clear. As end product of mixed-acid fermentation, formate is exported from the cytoplasm to periplasm where, in the presence of terminal electron acceptors, it serves as a substrate of the periplasmic formate dehydrogenases. In the absence of terminal electron acceptors it is re-imported to the cytoplasm to produce H_2. H_2 evolving activity in focB mutant grown on glucose, in glucose supplemented assays, at pH 7.5 was determined to be ~1.6 fold lower than in wild type. No difference was detected for focA mutant. To understand the role of formate in wild type H_2 producing activity, in the assays external formate at concentration of 10 mM was added. In the assays with formate H_2 evolving activity of focA mutant was similar, but in focB mutant it was ~2 fold lower than in wild type. Taken together these results have shown FocB activity during glucose fermentation in both assays. Moreover, no role of FocA in H_2 producing activity was observed. This might be due to that formate would be imported into the cell via FocB but not FocA channel. The low level of H_2 production rate in focB mutant in glucose and formate assays would result in sufficiently lower level of formate production in cytoplasm and due to some interaction between foc and pyruvate formate lyase (pfl) genes in the transcriptional level; the latter has been also suggested by the other group [2].

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The critical role of Na^+/Ca^{2+} exchanger on the maintenance of T-tubule structure

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Cardiac Na^+/Ca^{2+} exchanger (NCX1) is the primary Ca^{2+} extrusion system in beating myocytes, essential for Ca^{2+} homeostasis, and important in Ca^{2+} handling during excitation–contraction (E–C) coupling. NCX1 is specially localized in T-tubule and can be close to dyad, where is a predominantly E–C coupling take place. Therefore, T-tubule disorganization is linked to decreased contractility in heart failure (HF). Despite of T-tubule remodeling be correlated with Ca^{2+} handling defects in failing hearts (FH), the molecular mechanism has remained unclear. To examine whether the alteration of NCX1 expression and activity relate to the disorganization of T-tubule structure in FHs, we generated novel transgenic mice expressing NCX1 cardiac-specifically and inducibly, and examined the effect of inducing NCX1 expression during the progression of HF. We followed changes in NCX1 activity and expression during HF progression over 16 weeks in these mice, after transverse aortic constriction (TAC)-surgery. In TAC hearts, NCX1 activity increased over the first few weeks, but started to drop from 8 weeks after TAC before the onset of T-tubule disorganization and myocyte contractile dysfunction, which are common features in failing myocytes. Over the progression of HF, the expression of junctophilin-2 located at T-tubule/sarcoplasmic reticulum (SR) junction was gradually reduced in TAC hearts. Inducing NCX1 expression

restored NCX1 activity to the levels in pre-TAC, in 11 weeks TAC hearts. Maintaining NCX1 activity prevented progression of chamber dilation and cardiac dysfunction, even under prolonged pressure overload or long-term isoproterenol administration. In these myocytes, irregular T-tubule structure, abnormal intracellular Ca^{2+} handling, Ca^{2+}-overload of the SR, and contractile function were all impaired. In addition, the reduction of junctophilin-2 protein expression was preserved by inducing expression of NCX1 in TAC hearts. These data suggested that depressed NCX1 activity accelerated the progression of HF, while upregulating NCX1 is a potential therapeutic strategy to prevent HF. Taken together, this study suggests that maintaining local Ca^{2+} concentrations in dyadic clefts is crucial for cardiac structure and function in which NCX1 has a pivotal role.

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Comparative effects of single Cu(II) and Fe(III) ions and their mixture on *Enterococcus hirae* growth and membrane associated ATPase activity

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It is known that several heavy metals are called “essential” (iron, manganese, copper) and required for bacterial metabolism in small quantities. At the same time high concentrations of even such “essential” metals become toxic to microorganisms. Emissions of heavy metals to the environment are increased by many times during last years because of human activities. Microorganisms are exposed to a mixture of different heavy metals in the environment. In this respect it is interesting to examine effects of single heavy metals and to compare with results detected with mixtures of these metals. It is known that oxidizer Fe^{3+} and Cu^{2+} markedly affect *Enterococcus hirae* growth and membrane activity and the effects were opposite [1,2]. In the presence of 0.05–1 mM Fe^{3+} the growth and ATPase activity was increased even together with of N,N′-dicyclohexylcarbodiimide (DCCD), specific inhibitor of the FoF1-ATPase. Such results can be explained by existence of Fe-dependent ATPase in *E. hirae* which is active even in the presence of DCCD [2]. Cu^{2+} within the same concentration range inhibited *E. hirae* growth and ATPase activity with and without DCCD. These results indicate that Cu^{2+} might directly affect FoF1 ATPase thus modifying its activity [1]. At the same time we have established that simultaneous addition of these ions to bacterial growth medium caused effects which differ from the effects when the metals were added separately. We have shown that 0.1 and 1 mM Cu^{2+} and Fe^{3+} (metals were added in equal quantities) decreased the growth of *E. hirae* by increasing lag phase duration and decreasing specific growth rate. This mixture affected ATPase activity either and the inhibitory effect was detected even in the case when only metal ions were present (without DCCD). The effects had concentration-dependent manner. Surprisingly the effects detected when Fe^{3+} were added separately disappeared when two heavy metals were added simultaneously. Such effects might be a result of competing processes between Fe^{3+} and Cu^{2+}. The mechanisms of neutralization of the effects are still not clear.

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S10.P26

Computational studies of ion binding in cation/proton antiporters

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Na^{+}/H^{+} antiporters are essential for controlling cytosolic pH and salt concentration. As a critical element of the transport mechanism of Na^{+}/H^{+} antiporters, we focus on their ion binding properties. By using available structures and applying quantum-chemical methods, we are able to identify stable ion coordination geometries and protonation states. Detailed information like the optimal ion position, binding distances, and binding stabilities of different ions, are obtained. Additional atomistic molecular-dynamics simulations of membrane embedded antiporters provide a dynamic picture of the ion binding process. The simulations also quantify the water accessibility of the ion binding sites. Calculations shed light on the stability of the ion bound states, the role of buried charged residues, possible proton pathways, and transport mechanisms.

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Novel platform for single molecule analysis of membrane transporter activity by using arrayed lipid bilayer chambers

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The membrane transporters that transport substrate molecules across bio-membranes play pivotal roles for cell physiology, such as nutrient uptake, secretion of signal molecules, and energy transduction [1]. Although extensive studies have been conducted to elucidate the working mechanism of transporters, quantitatively measuring their transport activity remains difficult due to the complexity of the process to form membrane systems that allow the measurement of transporter activity in a reproducible and high-throughput manner. In the present study, we report an arrayed lipid bilayer chamber system (ALBiC) that displays a sub-million femtoliter chambers, each sealed with a stable lipid bilayer membrane with extremely high efficiency (yield: ~99%). When reconstituted with a limiting amount of the membrane transporter proteins, α-hemolysin [2] or FoF1-ATP synthase [3], the chambers of the ALBiC exhibited stochastic and quantized transporting activities, demonstrating that the single molecule analysis of passive and active membrane transport is achievable with the ALBiC system. Thus, this new platform could contribute to measure membrane transport activities with high sensitivity and throughput, and holds promise for understanding the working mechanism of membrane transporters.

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